

Increasing Speed of UHPLC-MS Analysis Using Single-stage Orbitrap Mass Spectrometer

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Overview

Purpose: Improve the performance of Orbitrap HR/AM systems for high throughput sample analysis

Methods: Full scan / all ion fragmentation MS analysis of complex samples in combination with UHPLC sample separation

Results: Significant increase of data quality and processing time could be accomplished with second generation MS hardware and processing software

Introduction

Productivity of a liquid chromatography-mass spectrometry (LC-MS) system is measured in samples per day. To accomplish high productivity, modern ultrahigh pressure LC-MS (UHPLC-MS) methods increasingly deal with very short gradients, leading to chromatographic signals with peak widths below 5 seconds at the base. It is still a challenge for high-resolution, accurate mass (HR/AM) systems to provide a sufficient number of scans (≥ 10) across the chromatographic peak in full scan mode without compromising sensitivity and selectivity. As reported earlier, a resolution in excess of 50,000 (FWHM@ m/z 200) is necessary to ensure a selectivity comparable to established MS/MS techniques¹, combined with a mass extraction window of 5 ppm. With this work we show data acquisition and processing by using the capabilities of the Thermo Scientific Exactive Plus mass spectrometer in combination with Thermo Scientific ExactFinder 2.0 processing software.

Methods

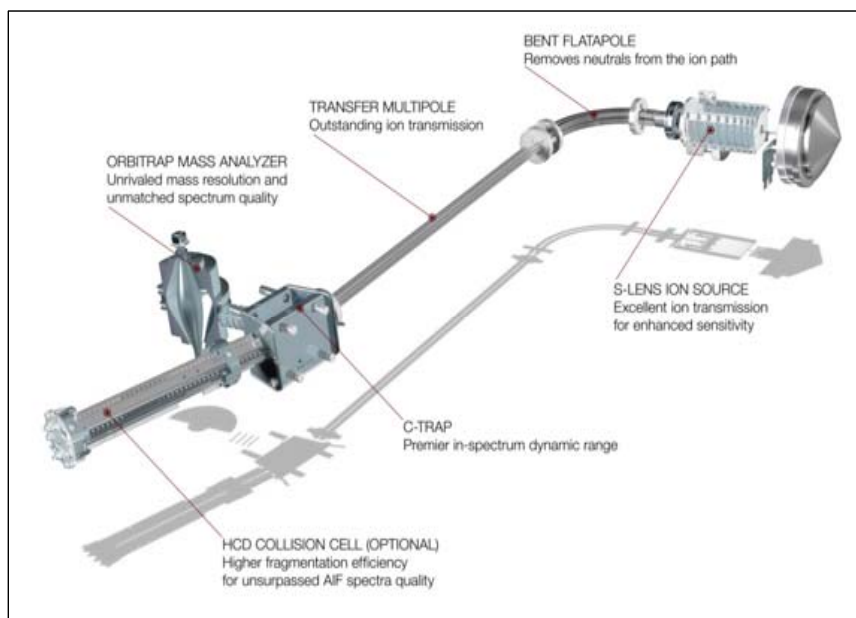
Sample Preparation: Quick, easy, cheap, effective, rugged, and safe (QuEChERS) extracts of horse feed were taken and spiked with common pesticides.

Liquid Chromatography (or more generically Separations): A Thermo Scientific Accela UHPLC system was used, consisting of an Accela™ open autosampler in combination with an Accela 1250 UHPLC pump. For analyte separation, a Thermo Scientific Hypersil GOLD PFP column (50 x 2.1mm, 1.9 μ m particle size) was used and a flow rate of 800 μ L/min was applied to generate a 2 min gradient (5 min full chromatographic cycle) of water and methanol, both spiked with 0.1% formic acid.

Mass Spectrometry: An Exactive™ Plus mass spectrometer was operated at 70,000 resolving power (FWHM) with full scan / data dependent AIF (FS / ddAIF) setting to generate all ion fragmentation scans based on an inclusion list containing the masses of the spiked components. The schematic of the Exactive Plus mass spectrometer is illustrated in Figure 1.

Data Analysis: The same data set was used for quantitative and qualitative data processing with ExactFinder™ 2.0 software. Qualitative processing included targeted screening in combination with general unknown screening.

FIGURE 1. Exactive Plus schematics



Results

Method Improvements: In the new Exactive Plus MS, two major changes have been implemented for performance improvement. First, the tube-lens / skimmer assembly has been replaced by the S-Lens, which serves for significantly higher ion transmission, increasing the instrument's sensitivity. Second, the assembly and the electronics of the Thermo Scientific Orbitrap mass analyzer have been improved², resulting in higher scan speed and resolution, together with improved pos / neg switching performance. As a result, the range of resolution is from 17,500 to 140,000 @ m/z 200, with a maximum scan rate of 12 Hz. Given that a resolution in excess of 50,000 is needed, the system was set to a resolution of 70,000 @ m/z 200, resulting in a scan rate of 3.7 Hz. As shown in Figure 2, for a 3.2 seconds wide peak, 13 scans across the peak were achieved. For improved component identification, it would be desirable to have fragmentation scans on the analytes of interest, but permanent switching between full scan and fragmentation scan mode (Full Scan / AIF) would decrease the data rate significantly, leading to the need to reduce the resolution setting. As a compromise, data dependant AIF scans were introduced into the full scans (FS / ddAIF) by means of a mass inclusion list, on which only one AIF scan for each target compound is triggered as soon as it crosses a given intensity threshold in a full scan. By means of this, the number of fragmentation scans is significantly reduced, keeping the overall data rate close to full scan only mode. Method details are shown in Figure 3. The Exactive Plus MS is shown in Figure 4.

FIGURE 2. Zoom on peak of Clofentazine.

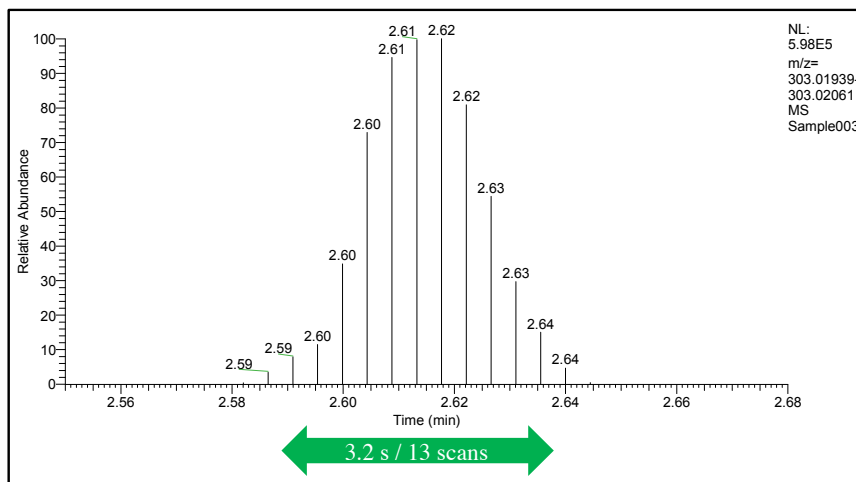
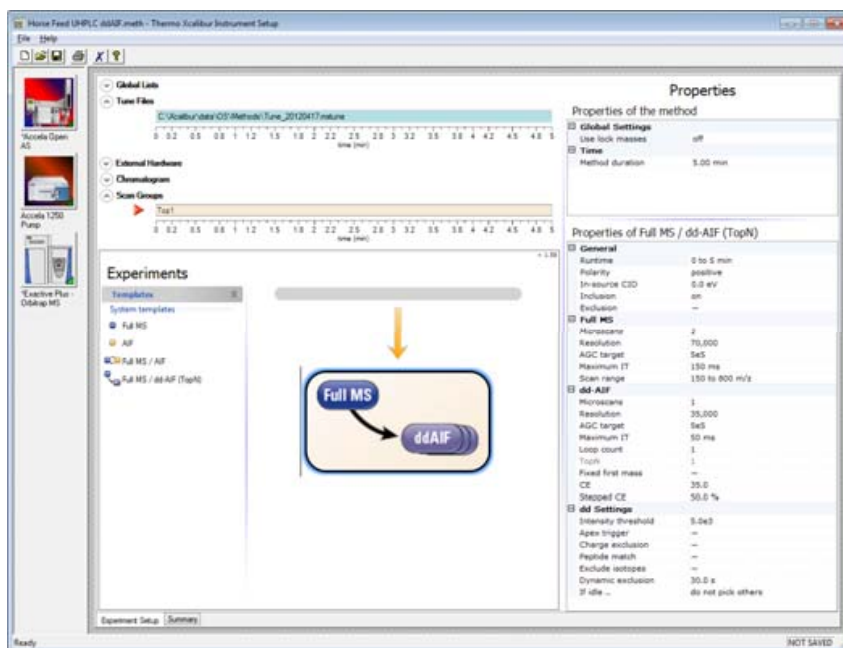


FIGURE 3. Exactive Plus Instrument Method.

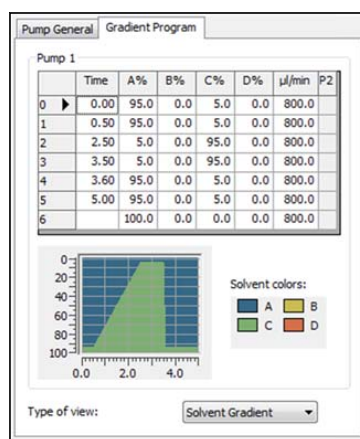


Sample Analysis: For chromatographic separation, a 2-minute chromatographic gradient was applied to QuEChERS extracts of horse feed samples spiked with pesticides, resulting in a total chromatographic cycle time of 5 minutes. 10 μL of each sample were injected onto a Hypersil GOLD™ PFP column (50 x 2.1 mm, 1.9 μm particle size) with a flow rate of 800 $\mu\text{L}/\text{min}$ (see Figure 5). This resulted in peak widths of 3 to 6 seconds for the analytes of interest. For targeted analysis, 85 commonly occurring pesticides out of the list of spiked components were chosen, using built-in databases from ExactFinder software. This selection could be exported directly into an Exactive Plus inclusion mass list file used by the Exactive Plus method for the ddAIF scan triggering as described previously. No further optimization of the LC-MS system was needed.

FIGURE 4. The Exactive Plus MS with an Accela open autosampler and Accela 1250 UHPLC pump



FIGURE 5. LC gradient



Quantitative Analysis: Quantitative analysis was done on the described selection of 85 pesticides. The sequence measured contained a dilution series with five samples of spiked matrix ranging from 5 $\mu\text{g}/\text{kg}$ to 150 $\mu\text{g}/\text{kg}$ spike level. As to be expected, the majority of compounds eluted in little more than one minute, so already a great number of target components were co-eluting (see Figure 6), not to speak of matrix components. However, the extracted ion chromatograms of most target components were free from additional peaks, showing that the selectivity of a 5 ppm extraction window in combination with the resolving power of the mass spectrometer proves very high selectivity. A linear calibration curve could be achieved for nearly all target components (example shown in Figure 7), giving additional hint that no significant matrix interference was present.

FIGURE 6. Extracted chromatograms for a selection of compounds

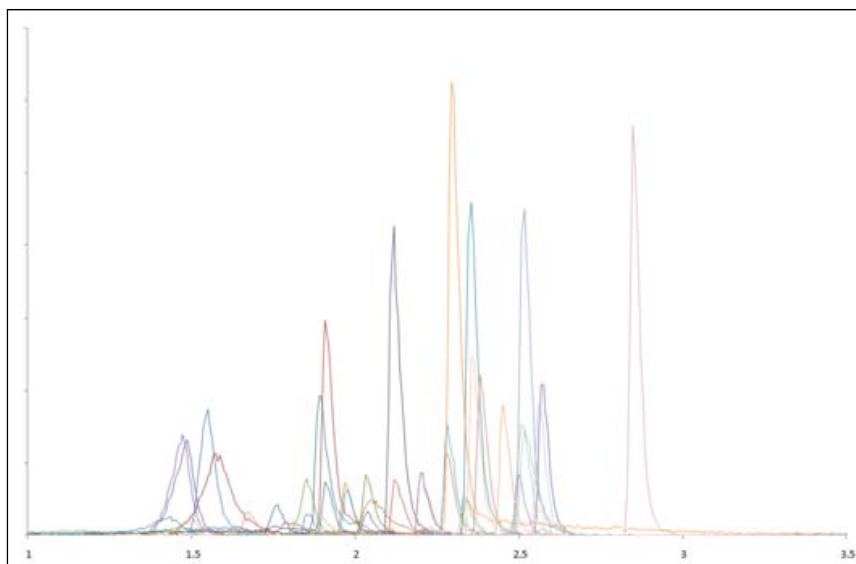
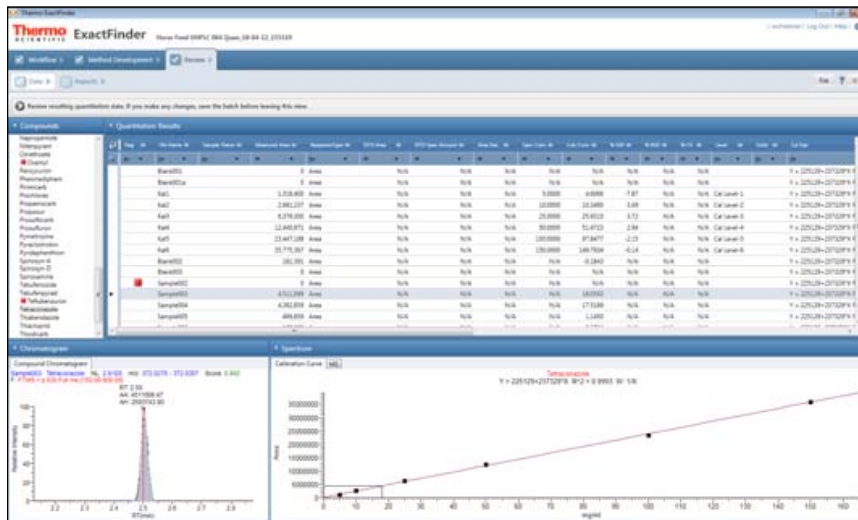


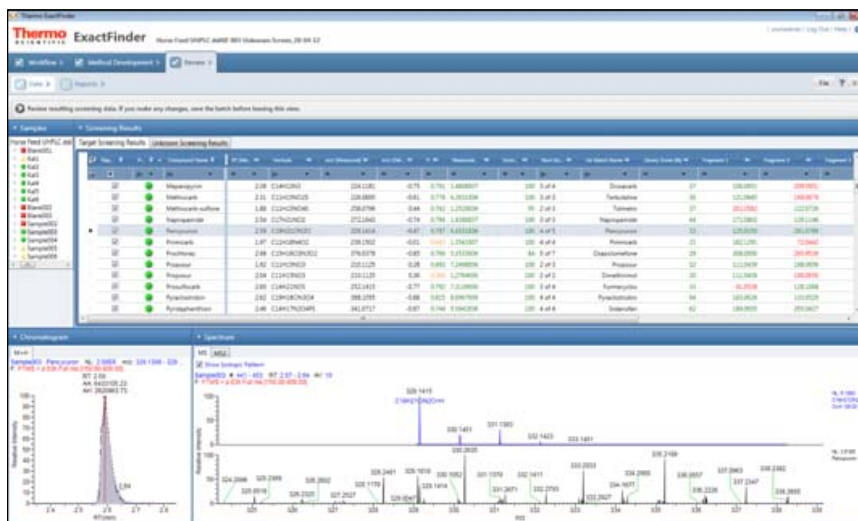
FIGURE 7. Quantitative Result Grid



Qualitative Analysis: Qualitative analysis was carried out as a combination of targeted analysis and general unknown screening. In this case, in a first stage a targeted analysis is carried out and in a second stage all peaks not identified in this targeted search are automatically forwarded to general unknown screening.

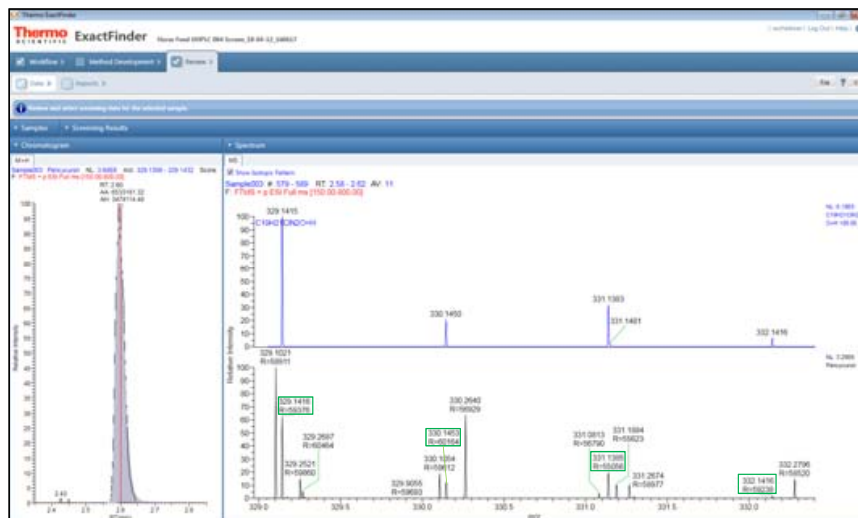
For the targeted search the same list of analytes as for quantitative analysis was applied. As confirmation criteria for targeted search retention time, isotopic pattern match, fragment search and library search were applied. The fragment information for the analytes of interest as well as the fragmentation spectra for the library search were taken from databases which are delivered with the ExactFinder software. Even at the lower end of the concentration range most components quantified could be easily confirmed on all four stages of confirmation (see Figure 8). The ExactFinder software provides easy and quick overview over the screening results with built-in reporting capabilities. It became clear very quickly that sufficient resolution is the key to success in full scan quantitation and screening. As shown in Figure 9, in complex samples like the ones analyzed here, most analyte signals are surrounded by numerous matrix signals. Only sufficient resolving power can ensure proper separation of analyte and matrix signals. This of course applies for the monoisotopic signals used for analysis as well as for the isotope signals used for confirmation. Looking at the fact that the peaks of interest show a resolution of close to 60,000, it becomes clear that significantly lower resolving power at this mass would lead to interference of these signals, causing significant mass shifts of the merged signals. As a consequence, this would lead to false negative results on the one hand, or it would require to widen up the extraction window, which would lower the selectivity of the analysis with the result of false positive results. Already the reduction of resolving power to 30,000 at this mass causes a shift of more than 2 ppm (results not shown).

FIGURE 8. Qualitative Result Grid



The general unknown screening carried out on the remaining peaks offers several options for automatic identification of the found peaks: database search, elemental composition determination based on isotopic pattern matching, library search and internet search. For the samples, roughly 15,000 components were detected which all went through the identification process. Database and library searches were carried out using built-in resources. Internet search was carried out using a selection of databases listed in the ChemSpider™ online search portal. A good number of additional contaminants could be identified, especially pesticides and a selection of Aflatoxins (results not shown).

FIGURE 9. Isotopic pattern match of Pencycuron. Green boxes mark the isotope signals surrounded by matrix signals



Conclusion

HR/AM analysis is a versatile method for residue analysis offering full quantitation capabilities in combination with unrestricted target and unknown screening options. Ultrahigh resolution delivered by the Orbitrap™ mass analyzer detection in the Exactive Plus mass spectrometer serves for reliability and selectivity comparable to established MS/MS techniques. Full UHPLC compatibility with uncompromised resolution and mass accuracy is shown with the new mass spectrometer.

References

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